Longitudinal assessment of oxaliplatin-induced neuropathy

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ABSTRACT

Objectives: To characterize the natural history of oxaliplatin-associated neuropathy (ON) and determine whether intraepidermal nerve fiber density (IENFD) is a sensitive measure of neuropathy progression. In addition, we sought to assess the potential of ON as a neuroprotection model and gain insight into the relationship between axon loss and neuropathic symptoms.

Methods: Eight subjects receiving oxaliplatin for advanced colorectal cancer were prospectively followed prior to starting chemotherapy and at 30, 90, 180, and 360 days (180 days after completing treatment). Electrophysiology, punch biopsies, symptom assessment, and examinations with calculation of a reduced total neuropathy score (rTNS) were performed at each time point. Changes over time were assessed through Poisson regression for IENFD and a mixed effects model for rTNS and electrophysiology measures.

Results: The distal leg IENFD, rTNS, peroneal, and sural amplitudes were all significantly reduced over time, while conduction velocity (peroneal and sural) and distal thigh IENFD were not. Measures of axon loss continued to worsen following discontinuation of oxaliplatin. Five of 8 subjects reported prominent symptoms associated with oxaliplatin administration.

Conclusions: This study demonstrates that oxaliplatin is associated with mild, sensory, and motor axon loss that may not be reversible. Axonal loss was detected by electrophysiology, rTNS, and distal leg IENFD. Several subjects reported prominent sensory symptoms that were not associated with axon loss, and that may or may not represent neuropathy. ON is an attractive paradigm for neuroprotection studies and the distal leg IENFD is an objective measure that requires minimal subject participation or study site expertise. ***Neurology*® 2011;77:980–986**

GLOSSARY

5-FU = fluorouracil; **IENFD** = intraepidermal nerve fiber density; **NCV** = nerve conduction velocity; **ON** = oxaliplatin-associated neuropathy; **PN** = peripheral neuropathy; **rTNS** = reduced total neuropathy score.

Oxaliplatin (Sanofi-Aventis; Bridgewater, NJ) is a third-generation platinum derivative that has enhanced inhibition of DNA repair and replication. Oxaliplatin is an antineoplastic agent currently indicated for the treatment of advanced cancer of the colon or rectum.[1] A dose-limiting toxicity of oxaliplatin is peripheral neuropathy (PN). As oxaliplatin-containing regimens become more successful, the number of long-term survivors will likely increase and neuropathy may emerge as a more important factor limiting quality of life.[1–5] In addition, "stop-and-go" dosing regimens have been adopted in an effort to reduce chronic neuropathy development[1,6,7] and underscores the importance of better understanding this neuropathy.

Unpleasant paresthesias in the distal extremities, mouth, and throat are common adverse events associated with acute oxaliplatin administration while a distal length-dependent neuropathy develops with total doses $\geq 540-850$ mg/m^2.[2,8]

The symptoms, functional impairment, and motor axon excitability[9] associated with oxaliplatin-associated neuropathy (ON) have been well described. We investigated the pathol-

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Table 1 Demographic features, oxaliplatin total dose, and number of cycles

Subject no.	Age, y	Sex	Weight, kg	Baseline TNS	Baseline distal leg IENFD	No. of cycles	Total doses, mg
1	44	M	69.4	4	19.0	6	780
2	52	M	82.5	0	21.0	7	1,437
3	83	M	68.5	7	14.5	10	1,133
4	70	F	43	1	19.5	6	516
5	45	F	81	0	5.9	12	1,950
6	55	M	81	7	8.1	3	522
7	52	F	69	0	24.7	11	1,498
8	75	F	61	1	10.2	12	1,448

Abbreviations: IENFD = intraepidermal nerve fiber density; TNS = total neuropathy score.

ogy of peripheral nerves in ON and rigorously assessed subjects longitudinally with traditional peripheral neuropathy assessments to determine which measures would detect neuropathy progression. Finally, we sought to assess the potential of ON as a model system to assess neuroprotective agents.

METHODS Eight subjects receiving oxaliplatin for advanced colon cancer were prospectively evaluated for neurotoxicity at John Hopkins Hospital, Baltimore, MD. Inclusion criteria required male or female subjects between 18 and 85 years of age with advanced (stage III) colon cancer requiring chemotherapy treatment. Subjects with prior exposure to neurotoxic agents including ethanol (>2 drinks/day), pyridoxine (>100 mg/day), Taxol, colchicine, allopurinol, or phenytoin; diagnosed diabetes, uremia, significant peripheral vascular disease, HIV, or progressive or degenerative neurologic disorders (e.g., multiple sclerosis, B12 deficiency); with history of lumbosacral laminectomy or radiculopathy; who were on antiepileptic medications; or who had established or suspected family history of inherited neuropathy were excluded from the study.

Subjects were evaluated prior to starting chemotherapy and at 30, 90, and 180 days following oxaliplatin initiation. A final assessment occurred 180 days after chemotherapy completion. No subject received radiotherapy. Nerve conduction testing, punch biopsies, and clinical examinations with calculation of a rTNS[10,11] were performed at each time point. The TNS is a validated peripheral nerve scoring system that encompasses subject symptoms, signs, and results of quantitative testing. The rTNS has been shown to have a high correlation with the full TNS (Spearman $r = 0.98$)[12] and differs in that it does not incorporate reports of motor symptoms, autonomic symptoms, or a quantitative vibration measurement. A trained neuromuscular specialist performed all examinations.

Nerve conduction studies were performed under a uniform protocol using an Oxford Instruments TECA Synergy EMG machine and included bilateral sural sensory responses and bilateral common peroneal motor responses. F-waves and EMG were not performed. The average of 10 antidromic responses was recorded for the sural nerve with the active electrode placed behind the lateral malleolus and the cathode 11 cm proximal in the calf. Supramaximal, single compound muscle action potential responses were recorded for the peroneal nerve with the active electrode placed over the belly of the extensor digitorum brevis muscle. Stimulation sites included the anterior ankle, immediately distal to the fibular head, and the popliteal fossa. A bar electrode was used for the recordings. Limb temperature was maintained at >32°C at the dorsum of the foot. An average of the bilateral nerve responses was used. The nerve conduction studies were performed by the same technicians and results of previous testing were not available during subsequent assessments.

Subjects underwent 3-mm punch skin punches at the lateral aspect of the distal leg and distal thigh; repeated biopsies were performed adjacent to the original biopsy sites using standard techniques.[13] A template was used to avoid biopsying a previous biopsy site. Specimens were fixed and stained with PGP 9.5 (ubiquitin hydrolase, Chemicon) and a trained, blinded technician assessed the intraepidermal nerve fiber density (IENFD) as described elsewhere[13] and compared to established normative data for our laboratory.[14] All subjects were recruited through the Johns Hopkins Kimmel Cancer Center and received oxaliplatin chemotherapy as part of a folinic acid (leucovorin), fluorouracil (5-FU), oxaliplatin (FOLFOX) regimen for metastatic colorectal cancer. The treatment protocol called for a 2-day regimen every 2 weeks for 12 cycles. Oxaliplatin was prescribed on day 1 at 85 mg/m^2 per cycle, combined with leucovorin 200 mg/m^2, a 5-FU 400 mg/m^2 IV bolus, and 5-FU 600 mg/m^2 IV as a 22-hour continuous infusion. Day 2 repeated leucovorin and 5-FU dosing.

Standard protocol approvals, registrations, and patient consents. The Institutional Review Board at John Hopkins University approved the study protocol. The Health Insurance Portability and Accountability Act of 1996 Privacy and Security Rules were followed during data collection and analysis. Subjects signed the informed consent after the nature of the study had been fully explained to them.

Statistics. Changes over time for the different neuropathy measures were assessed through mixed model regression for the IENFD and a mixed effects model for TNS and nerve conduction measures, thus accounting for longitudinal measurements within individuals. Continuous measures (neuropathy scores and nerve amplitudes) were modeled using mixed model linear regression, while IENFD was modeled as a Poisson variable because it is based on a count of nerve fibers within a sample volume. The effect of age, gender, total oxaliplatin dose, and number of cycles were assessed by adding these measures as covariates to the regression model. The average response over time for each measure was generated using the regression equation. All calculations were performed using Stata 10.0 (College Station, TX).

RESULTS Eight subjects were enrolled: 4 female and 4 male. The median age was 53.5 years, range 44–83 years. Subject demographics, total chemotherapy doses, and number of cycles are given in table 1.

All subjects were present at the initial and day 30 visits. Seven were seen at day 90 and day 180 while 4 returned at day 360. The subject who did not return for the day 90 visit dropped out of the study due to deteriorating health. Baseline, day 30, 90, and 180 visits were coordinated with subjects' oncology appointments and 3 subjects did not return for their day 360 evaluations, which were not associated with an oncology appointment. One subject (no. 3) returned for the day 360 visit and underwent skin biopsy testing and nerve conduction velocity (NCV)

testing but not an examination. Four subjects received abbreviated oxaliplatin treatment due to deteriorating health unrelated to any neurologic issue. Three of the 4 subjects who received 10–12 cycles were also assessed at the day 360 time point. No patient received radiation therapy.

Neuropathy symptoms. Subjects had relatively few autonomic or motor symptoms. When present, autonomic symptoms consisted of constipation or diarrhea that was attributed to subjects' underlying illness, and not to an autonomic neuropathy. Similarly, motor symptoms were a rare complaint and further justified the use of the rTNS.[15] The results did not change significantly if autonomic and symptoms of weakness were incorporated into the rTNS measure. Sensory symptoms were prominent and most commonly consisted of paresthesias in the hands, feet, and lips or mouth. Three subjects reported perioral tingling or laryngeal tightening with drinking cold liquids that were very bothersome. Two subjects reported distal limb paresthesias that were uncomfortable and interfered with buttoning clothes or made walking uncomfortable. No subject required symptomatic treatment with conventional neuropathic pain medications (such as gabapentin, duloxetine, tricyclic antidepressants); all subjects received Ca^{++}/Mg^{++} infusions as part of their oncologic protocol.

Neuropathy signs. Among the components of the neurologic examination performed as part of the rTNS, signs attributed to large fiber neuropathy (vibration sensibility and deep tendon reflexes) increased from 0.75 ± 1.42 points at baseline to 2.3 + 1.4 at day 180 and 2.7 ± 0.6 at day 360. Small fiber signs were limited to pin sensibility and were 0.4 ± 0.7 at baseline, 1.2 ± 1.2 at day 180, and 0 at day 360. Motor weakness on examination was 0 at baseline, 0.43 ± 0.53 at day 180, and 1.0 ± 1.4 at day 360.

Neuropathy measures. The results of the rTNS and IENFD scores at the different time points are presented as spaghetti plots in figure 1 and the electrophysiology results are given in figure 2. No subject had an abnormal baseline sural or peroneal response. Table 2 depicts the regression analysis results. All procedures were well tolerated by the study subjects and there were no infections associated with the punch skin biopsies.

The average rTNS values increased from a baseline of 2.5 ± 3.07 to 7.85 ± 3.48 at 180 days and 8.66 ± 4.16 ($p < 0.05$) at 1 year. The average distal leg IENFD decreased from 15.39 ± 6.75 at baseline to 12.89 ± 4.73 at day 180 and 9.45 ± 3.92 at day 360.

The distal leg IENFD, peroneal amplitude, sural amplitude, and rTNS were all significantly decreased over time while peroneal conduction velocity, sural conduction velocity, and distal thigh IENFD were not (table 2). Age, gender, cumulative dose, and number of cycles were not significant when added to the regression model individually though we recognize that the sample size was very small. Among the different measures, the change in rTNS value was the largest at a decrease of 0.02 units/day or a 2-unit decrease after 100 days and is consistent with the measure being a composite score. A change of 2 TNS points has been considered to be meaningful in other studies.[10,11]

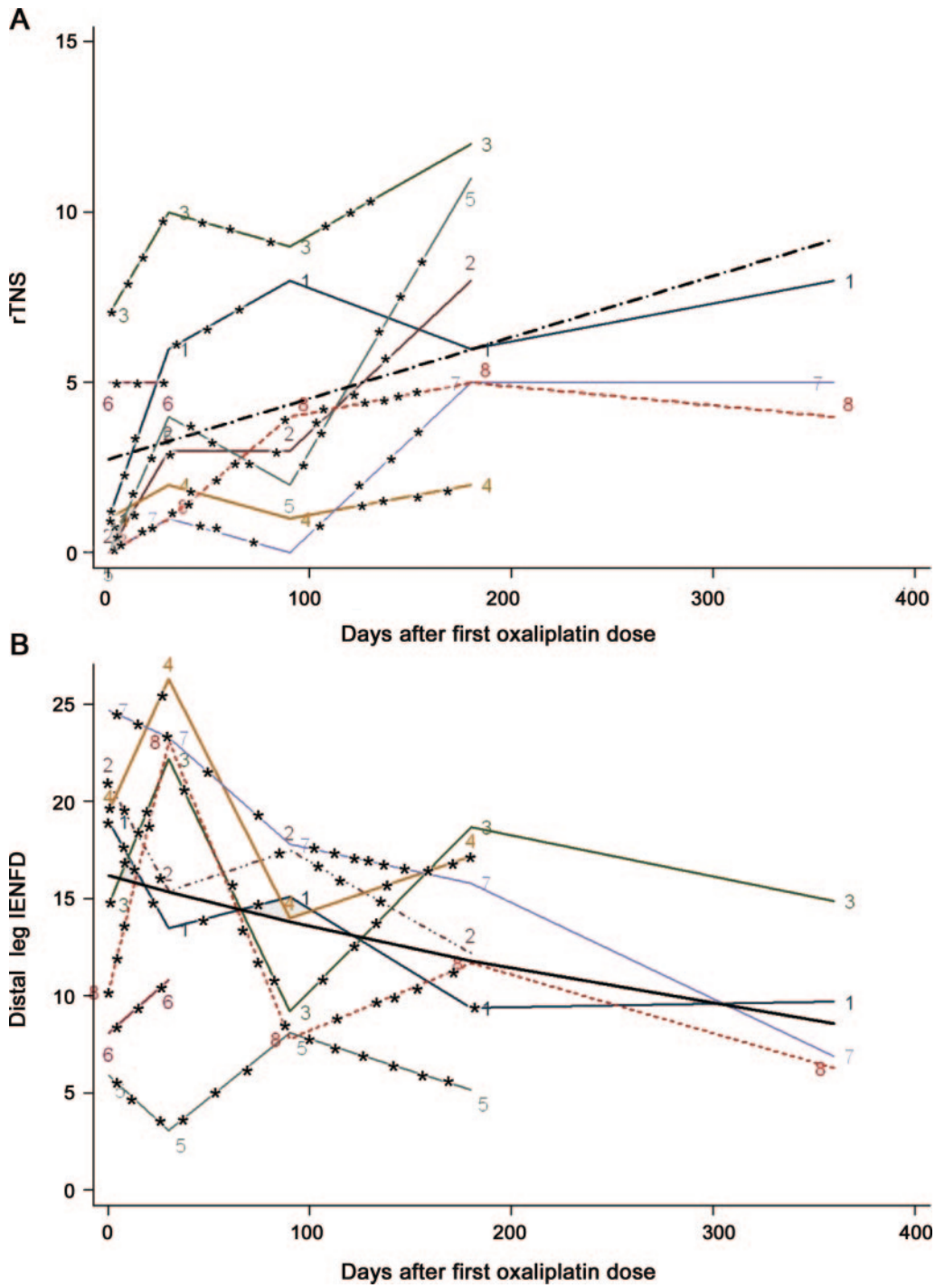
The change in IENFD at the distal leg was more pronounced than at the distal thigh IENFD. Subjects 1, 3, 7, and 8 had a skin biopsy at day 360, 6 months after their last dose of oxaliplatin. Three of these subjects (3, 7, and 8) had a further decrease in IENFD at day 360 compared to day 180 (20%, 57%, and 47% decrement in distal IENFD, respectively). Subject 1 did not show any decrement at day 360 and rather had a small increase.

DISCUSSION This study has several important findings. First, it shows that oxaliplatin administration is associated with mild, progressive, sensory > motor axon loss that can be detected using several accepted peripheral nerve measures. Second, our results suggest that axon loss can progress after cessation of oxaliplatin administration and that this may be irreversible. These results provide a rationale to include such a patient population in neuroprotection studies and have implications for the design of such studies. Finally, these results underscore the difficulty in distinguishing between neuropathy symptoms and axonal loss.

Several measures documented a significant decrease in nerve function over time including distal leg IENFD, rTNS, as well as the peroneal motor and sural nerve amplitudes. There was no change in nerve conduction velocity measurements or in the distal thigh IENFD. Of these different measures, the decrease in the peroneal nerve amplitude was the smallest and was subclinical. Sensory symptoms and signs as well as NCV abnormalities accounted for the majority of points in the rTNS. We did not observe any correlation between age, stage of tumor or other medical condition, and severity of peripheral neuropathy, though we recognize that our sample size is small.

Based on these findings, we conclude that oxaliplatin induces distal sensory and motor axon loss though the degree of loss was mild (no patient developed an abnormal sural or peroneal amplitude, or an IENFD below the fifth percentile).[14] Additionally, the significant decrease in the distal leg IENFD in

Figure 1 Spaghetti plots of reduced total neuropathy score (rTNS) (A) and intraepidermal nerve fiber density (IENFD) (B) at baseline, 30 days, 90 days, 180 days, and 360 days in each subject

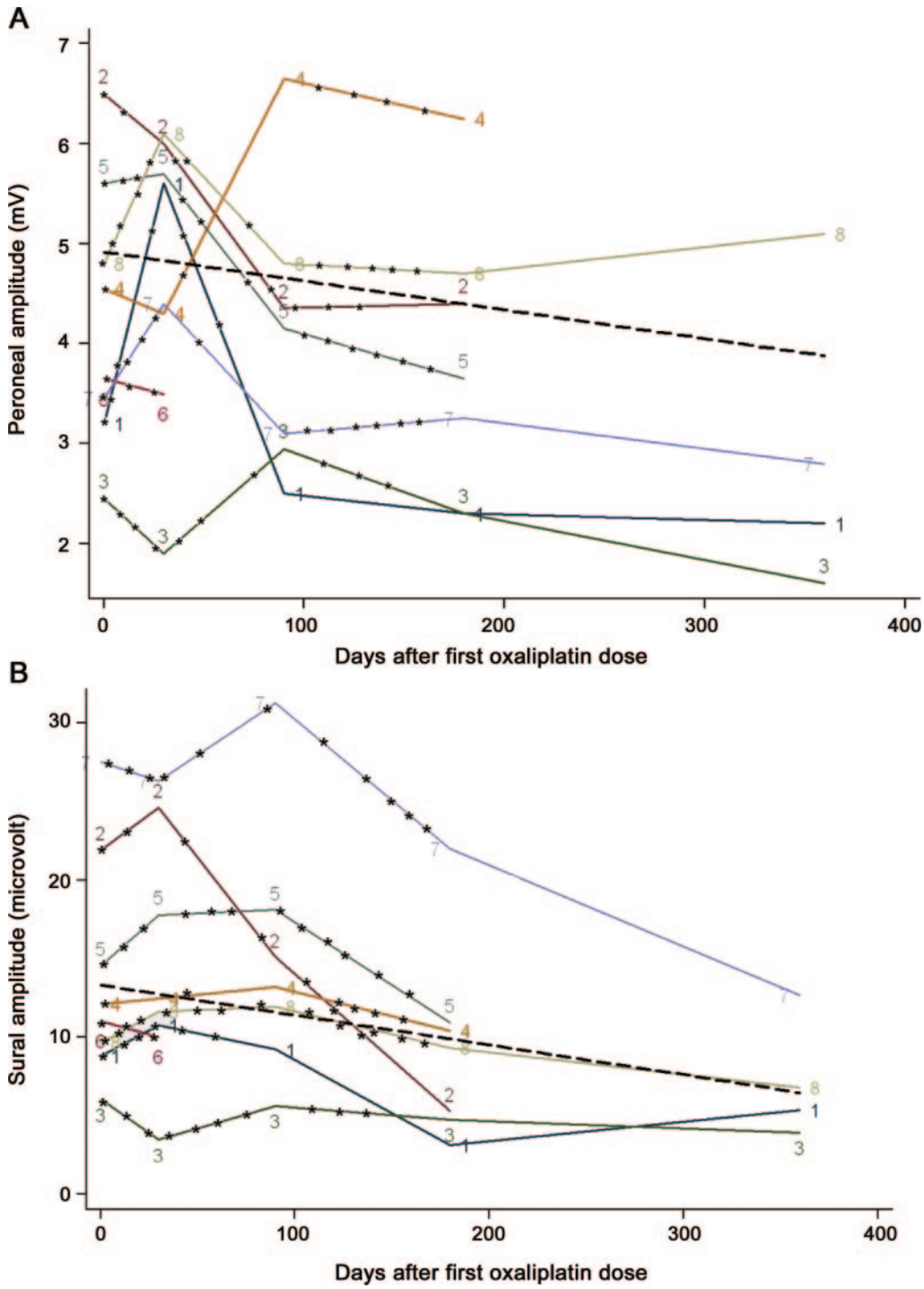


Numbers on graphs correspond to subject number in table 1. Dark black lines represent the predicted regression line for all data. Stars indicate dates of oxaliplatin administration.

the setting of no change at the distal thigh IENFD suggests that chronic oxaliplatin-induced neuropathy is a length-dependent process preferentially affecting the terminals of the longest axons. Patients with sensory ganglionopathies typically have IENFD reductions at both proximal and distal sites,[16] which was not observed in our study. While cisplatin is accepted to have dorsal root ganglion toxicity, these data imply that the toxicity was not detected at proximal sites.

These findings have several implications for future chemotherapy-induced neuropathy studies. First they imply that oxaliplatin-induced neuropathy is an attractive paradigm for neuroprotection studies as we were able to reliably measure axon loss by sev-

Figure 2 Spaghetti plots of peroneal (A) and sural nerve (B) amplitudes across time



Numbers on graphs correspond to subject number in table 1. Dark black lines represent the predicted regression line for all data. Stars indicate dates of oxaliplatin administration.

eral measures. Patients receiving chemotherapy are suitable for neuroprotection studies given that the timing of the toxic exposure is known and can be quantified. This advantage is partially offset by comorbidities in a patient population that often is chronically ill, on multiple neurotoxic medications, receiving different chemotherapy regimens, and subject to study fatigue toward ancillary studies. Objective measures of axon loss performed well in this study and appear to be well-suited to such studies. The skin biopsy procedure was well-tolerated and the distal leg was the best site to monitor axon loss. Additionally, skin biopsy is attractive as it is unbiased, quantitative, and requires limited subject time com-

Table 2 Regression analysis results of each measure for assessing degree of neuropathy

Measure	Coefficient	p	95% CI
Distal leg IENFD, fibers/mm	−0.00176	<0.001	−0.00266, −0.000853
Distal thigh IENFD, fibers/mm	−0.000333	0.455	−0.00121, −0.000541
Peroneal motor CV, m/s	0.0018	0.7	−0.000725, 0.0109
Peroneal motor amplitude, mV	−0.0032	0.02	−0.00587, −0.000536
Sural amplitude, μV	−0.277	0.005	−0.4717, −0.00816
rTNS	0.0179	<0.001	0.00914, 0.02674

Abbreviations: CI = confidence interval; IENFD = intraepidermal nerve fiber density; rTNS = reduced total neuropathy score.

mitment and no neurologic expertise by the person performing the biopsy. Furthermore, the progression of axon loss following the cessation of chemotherapy is consistent with the phenomenon of coasting and these data suggest that periods longer than 6 months may be needed before recovery can be detected. These results also imply that neuroprotection studies might increase the power to detect a drug effect by having a study duration that exceeds the period of oxaliplatin administration.

The WHO[17] and National Cancer Institute Common Toxicity Criteria[18] grading systems have both been used to detect neuropathy progression in oxaliplatin based regimens. These scoring systems are heavily weighted toward subject symptoms and are consistent with a focus on palliation in advanced colorectal cancer. Several of our subjects developed prominent symptoms that interfered with drinking, walking, or performing dexterity tasks, though these symptoms were not captured by the rTNS and were not associated with axon loss. This distinction between axon loss and neuropathy symptoms is similar to reports demonstrating that the pain associated with CTS does not correlate with the severity of electrophysiologic abnormalities.[19] In general, scoring systems that rely solely on neurologic examinations have performed poorly in documenting the severity of and following subjects' neuropathy.[20,21] Objective peripheral nerve measures, including IENFD, may therefore complement existing symptom and examination-based scales in ON.

Oxaliplatin is associated with mild sensory and motor axon loss that could be detected by several measures including rigorous NCV testing, rTNS, and a distal leg IENFD. These results also suggest that patients receiving oxaliplatin are an attractive population to assess potential neuroprotective agents and that such objective measures of peripheral nerve function complement existing symptom-based scales. A distal leg skin punch has potential as such a measure as it is well-tolerated, requires little subject participation, and is well-suited to multicenter trials that include sites with little neurologic expertise.

AUTHOR CONTRIBUTIONS

Dr. Burakgazi: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data, statistical analysis, study supervision. Dr. Messersmith: study concept or design, acquisition of data, study supervision. Dr. Vaidya: analysis or interpretation of data, statistical analysis. P. Hauer: drafting/revising the manuscript, acquisition of data, study supervision. Dr. Hoke: drafting/revising the manuscript, study concept or design. Dr. Polydefkis: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data, statistical analysis, study supervision, obtaining funding.

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DISCLOSURE

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